Journal of Applied Biology & Biotechnology Vol. 13(2), pp. 104-112, March-April, 2025
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2025.213846

Identification, antimicrobial activity, and mycochemicals of *Lentinus* spp. cultivated in papaya peel medium

Alivia Nur Maysaroh[1], Lucia Dhiantika Witasari[2], Sari Darmasiwi[1*]

[1]Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia.
[2]Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

ARTICLE INFO

Article History
Received on: August 01, 2024
Accepted on: November 13, 2024
Available Online: January 25, 2025

Key words:
Identification, antimicrobial, mycochemical, *Lentinus* spp., GC-MS.

ABSTRACT

Lentinus spp. (Family Polyporaceae) are widely studied for their nutritional and medicinal properties; however, research on their cultivation outside their natural habitat is limited. This study aimed to identify and examine the mycochemical contents and bioactivity of *Lentinus* spp. cultivated in papaya peel medium. Two different species of *Lentinus* spp. were collected from Indonesia and subjected to morphological and molecular analyses. Mushroom mycelia were sub-cultured on potato dextrose agar and then cultivated on papaya peel at 25°C ± 2°C for 20 days. Ethanol extracts of the harvested mycelium were analyzed for their antimicrobial activities and mycochemicals using qualitative assays and GC-MS. Results identified these species as *Lentinus squarrosulus* (LSQ) and *Lentinus sajor-caju* (LSJ). Only LSQ showed antibacterial activity against *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis,* and *Propionibacterium acnes* ATCC 6919. Mycochemical analysis revealed that LSQ contained terpenoids, with hexadecanoic acid methyl ester (10.85%) and 9,12-octadecadienoyl chloride (10.82%) and maltol (8.73%) as its major compounds. This investigation revealed the possible antibacterial activity of LSQ against *P. acnes*, which was rarely documented.

1. INTRODUCTION

Mushrooms are eukaryotic heterotrophic organisms that absorb nutrients and play an essential role in decomposing organic materials to make them available for use by plants and other organisms. Most mushrooms are saprophytes. Out of the 1.5 million species of macrofungi or mushrooms, only 6.7% have been described and are primarily documented in temperate zones; those in tropical areas with high diversity remain largely unexploited and underexplored [1,2]. In tropical countries, the estimated number of fungal species is approximately 80,000 of which 16,000 are macrofungi or mushroom species [3].

The macrofungus *Lentinus* belongs to the family Polyporaceae and is prevalent worldwide due to its diverse species and cultural requirements, particularly in tropical countries where the rich rainforests provide optimal conditions for its growth [4]. Well-known *Lentinus* spp. exhibit unique dietary and therapeutic advantages and have been utilized by native populations across the world for their functional edible uses. Some species, such as *Lentinus edodes* in China [5] and *Lentinus squarrosulus* (LSQ) in the Philippines [6], are widely cultivated as food. *Lentinus* spp. are also rich in mycochemicals, including alkaloids, terpenoids, and flavonoids, which exhibit antibacterial activity against various bacteria, such as *Staphylococcus aureus, Bacillus cereus, Escherichia coli,* and *Pseudomonas aeruginosa* [7]. Further analysis using GC-MS identified several secondary metabolites from *L. squarrosulus*, including methyl linoleate, hexadecanoic acid methyl ester, methyl 2-oxo-1-pyrrolidine acetate and 9,12-octadecadienoic acid ethyl esters (linoleic acid ethyl ester), all of which possess antibacterial, anti-oxidant, anti-hypercholesterolemic and pesticide activities [8].

Despite their widespread distribution and potential for biological activity, only a few studies have focused on the cultivation of *Lentinus* spp. mycelium outside their natural habitat. In the present work, *Lentinus* spp. was collected from tropical areas in Indonesia were cultivated through solid-state fermentation (SSF) by employing organic agro-industrial waste such as fruit peel to produce many bioactive compounds [9]. A previous study used papaya peel as a medium for cultivating *Panus tigrinus* (Polyporaceae) and was reported greater mycelial biomass production than using banana or pineapple peel medium [10]. Papaya peel was also applied as growing medium for *Russula* and *Pleurotus* mushrooms, and all the mushroom cultures exhibited good biomass production [11]. Papaya peel contains 54.48% total carbohydrate, 10.56% protein, 54.48% water, 0.23% fat,

*Corresponding Author
Sari Darmasiwi, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia.
E-mail: saridarma @ ugm.ac.id

and 5.25% ash, rendering it a potential substrate for mushroom growth and a good source of bioactive compounds [12].

The cultivation and antimicrobial activity of *Lentinus* spp. in papaya peel medium has never been reported and must be documented. Therefore, this study aimed to identify and examine the mycochemical content and bioactivity of *Lentinus* spp. cultivated by SSF using papaya peel as the medium.

2. MATERIALS AND METHODS

2.1. Chemicals and Materials

Potato dextrose broth (PDB) and Mueller–Hinton Agar (MHA) media were obtained from HiMedia (India). Sabouraud dextrose agar (SDA) was purchased from Merck (USA). Nutrient agar (NA) and nutrient broth (NB) were acquired from Oxoid (UK). DNA extraction and purification kits were purchased from FavorGen (Taiwan), PCR mix and agarose gel were obtained from ABclonal (USA), and the ladder for electrophoresis was bought from PCRBIOSYSTEMS (UK). Loading dye was sourced from GeneDirectX (USA). As a medium for the SSF of mushroom, California (Callina) variety papaya was purchased from a local supermarket.

2.2. Mushrooms Sampling

Mushrooms were collected from two provinces: the forest surrounding Suak Gual Village in Belitung, Indonesia (2°54'49 "S 107°23'13 "E) and the area under dead wood around rice fields in Sleman District, Yogyakarta, Indonesia (7°44'18 "S 110°30'06 "E). The samples were stored in zip-lock plastic bags, sun-dried, and kept at 4°C in the laboratory for further analysis.

2.3. Morphological Identification of Mushrooms

The morphological identification of the mushrooms involved macroscopic observations and species identification based on their basidiomes (fruiting body) [13,14].

2.4. Mycelium Preparation

The mushroom basidiomes were cleaned under running water and then cut into pieces. The samples were treated with a 10% sodium hypochlorite solution and rinsed with sterile distilled water. The partially dried basidiomes were then inoculated on PDA medium and incubated for 3–7 days at room temperature (25°C ± 2°C) to observe mycelial growth [15].

2.5. Molecular Identification of Mushrooms

DNA was extracted from the basidiomes using the FavorPrepTM Plant Genomic DNA Extraction Mini Kit from FAVORGEN (Favorgen, Taiwan) following the manufacturer's guidelines. The isolated DNA was then amplified using PowerPol 2x PCR Mix with Dye from ABclonal (Massachusetts, USA). The PCR reaction mixture comprised 25 µl of PowerPol 2x PCR Mix with dye, 1 µl of ITS-1 primer (10 pmol/µM), 1 µl of ITS-4 primer (10 pmol/µM), 50 µl of nuclease-free water, and 8 µl of DNA template. The PCR amplification process consisted of the following steps: pre-denaturation at 94°C for 5 minutes, template DNA denaturation at 94°C for 30 seconds, annealing at 55°C for 45 seconds, DNA extension at 45°C for 2 minutes, DNA polymerisation at 72°C for 1 minute and a final cooling stage at 4°C. The amplification cycle was repeated 30 times. The PCR products were then run in electrophoresis using 1% agarose gel in TAE buffer. Purification was performed with FavorPrep GEL/PCR Purification Mini Kit (Favorgen, Taiwan), followed by sequencing (Genetika Science, Jakarta, Indonesia). The sequence results were then analyzed and compared with existing data in GenBank using the BLASTn tool from the National Center for Biotechnology Information (NCBI). Phylogenetic construction was performed using MEGA 11.0 (Pennsylvania State University, Pennsylvania, USA).

2.6. Mushroom Cultivation on Papaya Peel Medium by SSF

The papaya peel waste was thoroughly washed, cut, placed in an Erlenmeyer flask at a total weight of 20 g, and added with 2 ml of sterile distilled water. The medium was autoclaved and then allowed to cool for 24 hours before being aseptically inoculated with mycelium using a cork borer. Five plugs of mycelium (5 mm^2) were evenly distributed within the papaya peel medium. The inoculated medium was then incubated for 20 days at room temperature (25°C ± 2°C) until it was completely colonized by the mycelium [11].

2.7. Sample Extraction

The mushroom mycelia grown in papaya peel medium were harvested, soaked in 96% ethanol 1:2 (w/v), macerated for 72 hours, filtered with Whatman paper, concentrated with a rotary evaporator, and finally dried. The extract was then kept at 4°C and used for mycochemical and bioactivity analysis [15].

2.8. Antimicrobial Assays

The extracts were prepared by dissolving them in 0.5% DMSO (1 g/ml). Six bacterial strains, *Staphylococcus aureus* ATCC 25923, *Staphylococcus hominis* InaCC B226, *Staphylococcus epidermidis, Propionibacterium acnes* ATCC 6919, *P. aeruginosa* ATCC 27853, and *E. coli* 25922, were used as the tested bacteria, and *Candida albicans* was used as the tested fungus. The tested bacteria were sub-cultured in NA medium and grown in NB medium 24 hours prior to testing. The bacterial cultures in NB were adjusted to the 0.5 McFarland standard and then swabbed onto sterile Petri dishes containing MHA medium. Meanwhile, *C. albicans* was grown on PDB medium for 48 hours prior to testing and then swabbed onto sterile Petri dishes containing SBA medium. Subsequently, 10 µl of each extract was applied to a disc and placed in a circle on the MHA medium containing the tested bacteria and the SBA containing the tested fungus. The plates were then incubated at 37°C for 20–24 hours for bacterial growth and at 30°C for 48–56 hours for fungal growth. The diameter of the clear zone around each disc was measured to assess each extract's ability to inhibit microbial growth [16].

2.9. Mycochemical Analysis

Mycochemical testing included triterpenoids, tannins, alkaloids, saponins, and flavonoids.

2.9.1. Alkaloid test

For alkaloid testing, 5 ml of the extract was combined with 10 ml of MeOH and 10 ml of 2N HCL and then treated with Wagner reagent. Positive results are indicated by turbidity in the sample [17].

2.9.2. Terpenoid test

Terpenoid testing was performed using the Salkowski test. Initially, 5 ml of the extract was mixed with 2 ml of chloroform and then carefully

layered with 3 ml of sulphuric acid. Positive results are characterized by a red–brown coloration [18].

2.9.3. Tannin test

Tannin was detected using a $FeCl_3$ (ferric chloride) reagent. Briefly, 10 ml of the ethanol extract was combined with 5 ml of 5% ferric chloride solution. Color changes in the solution to grey or black indicate the presence of tannin compounds in the extract [19].

2.9.4. Saponin test

Saponin testing involved diluting 1 ml of the extract with 20 ml of distilled water and shaking the mixture in a cylindrical measuring cup for 15 minutes. The formation of a foam layer (approximately 1 cm thick) indicates the presence of saponin [20].

2.9.5. Flavonoid test

Flavonoid testing utilized a cyanidin reagent. Extracts containing flavonoids will produce a purplish color [21].

2.10. GC-MS

The extract was dissolved in a 1:1 (g/v) solution with 96% ethanol. The GC-MS instrument used in this research is the Agilent 7693A Autosampler equipped with the following specifications: a capillary column type HP-5MS UI (Agilent) (30 m × 250 µm × 0.25 µm). Helium was used as the carrier gas, the injector temperature was set at 250℃ and the injection volume was 1 µl. The split ratio was 01:10 with a split speed of 1 ml/minute, and the initial temperature was maintained at 50℃ for 1 minute. The temperature ramp began at 50°C for 1 minute, followed by an increase of 5°C/minute to 100°C for 3 minutes, then 5°C/minute to 150°C for 3 minutes, 5°C/minute to 250°C for 3 minutes and finally 10°C/minute to 280°C for 5 minutes. The detector employed was the Agilent 5977B Mass Spectrometry with a mass range of 30–550. The obtained results include a table of peak chromatograms and compounds [22].

2.11. Data Analysis

The results of the triple experiments are shown as the mean and standard deviation (SD). Antimicrobial data were analyzed with SPSS software (SPSS Inc., Chicago, IL, USA). One-way ANOVA was conducted, followed by a DMRT post-hoc test for significant differences ($p < 0.5$). Phylogenetic analysis was performed using MEGA 11.0 software with the maximum likelihood (ML) method and 100 repetitions [23].

3. RESULTS AND DISCUSSION

3.1. Mushroom Identification

The two *Lentinus* samples were isolated from dead trees in the tropical forests of Belitung Islands and from dead wood near rice fields full of litter in the Special Region of Yogyakarta Province. They were labeled B1 and Y1 and identified based on their morphological characteristics.

Both mushrooms belong to the order Polyporales because they have distinctive pores. This order consists of various types of basidiocarps, such as bracket-shaped basidiocarps (genus *Fomitopsis*), flat-shaped (genus *Lentinus*), smooth-shaped (genus *Podoscypha*), hymenophore with a poroid (genus *Perenniporia*) and resuspended to effusion shaped-reflection (genus *Ceriporiopsis*, *Phlebia*). The mushroom samples exhibit a distinct morphology, featuring light brown to brown scales, serrated edges, and white to yellow underside lamellae. They grow individually or in groups on wet wood, logs, and tropical tree stumps, are extensively found in tropical regions, and can withstand a broad range of temperatures. These characteristics are typical of the genus *Lentinus* [4,24].

Fungi can be identified using morphological and molecular methods. Morphological differences in color, surface texture of their caps, attachment of the cap to the stalk, spacing of gills, and stalk shape of the basidiomes between the mushroom samples were observed to determine their species (Fig. 1).

According to morphological observations, the B1 sample was suspected to be *L. squarrosulus* due to the presence of scabs, scales, or crusts, which are critical features of this species [24]. *L. squarrosulus* basidiocarp has a slightly creamy white color, a rough hood surface, and a slightly brown center. It has a pileus diameter of 2.7–5.5 cm and lamellae with a size of 1.2–4.7 cm attached to the stalk with shortly decurrent (shortened lamella length) and moderate inter-row lamella characters. The stipe is 1.8–3.5 cm long with a cylindrical shape (uniform stalk size from tip to stalk) as described in their identification key [13,14]. Meanwhile, the Y1 sample was suspected to be *L. sajor-caju*. *L. sajor-caju* basidiocarp is yellow-brown with a smooth, fibrous, and fleshy hood surface, a pileus diameter of 4.5–5.8 cm. The lamellae have a size of 3.9–4.5 cm and are attached with a vast distance (adnate); the spacing between lamella rows is tight. The stipe is 1.5 cm long with a root-like shape. The primary identification characteristic of *L. sajor-caju* is the presence of an annulus. However, no annulus was found in the Y1 sample possibly due to genetic variations, suggesting that *L. sajor-caju* typically develops pseudoannula upon maturation [25]. Additionally, the sample underwent color changes or was hygrophanous, transitioning from white to yellow-brown, further supporting its classification as *L. sajor-caju* [26].

Molecular identification was also carried out to ensure accurate species identification based on DNA sequences. The ITS region was utilized for molecular identification to identify the fungal species [27]. The morphological identification was corroborated by the molecular data obtained using the ITS-1 and ITS-4 primers for Basidiomycota fungi, which included the 5.8S rRNA gene, ITS-1 and ITS-2 regions [28]. The results showed a band with a length of approximately 600 bp for both mushroom species. BLAST analysis revealed that the mushroom isolated from Yogyakarta (Y1) shared 97% similarity and 100% query coverage with the *L. sajor-caju* species and was submitted to NCBI as *L. sajor-caju* PP236948.1. Meanwhile, the mushroom isolated from Belitung Islands (B1) exhibited 93% similarity and 94% query coverage with the *L. squarrosulus* species and was submitted to NCBI as *L. squarrosulus* PP211943. 1. A phylogenetic tree was constructed using the ML method as shown in Figure 2.

Figure 1. Front and back views of (a) *Lentinus sajor-caju* (LSJ) from Yogyakarta and (b) LSQ isolated from Belitung Province, Indonesia.

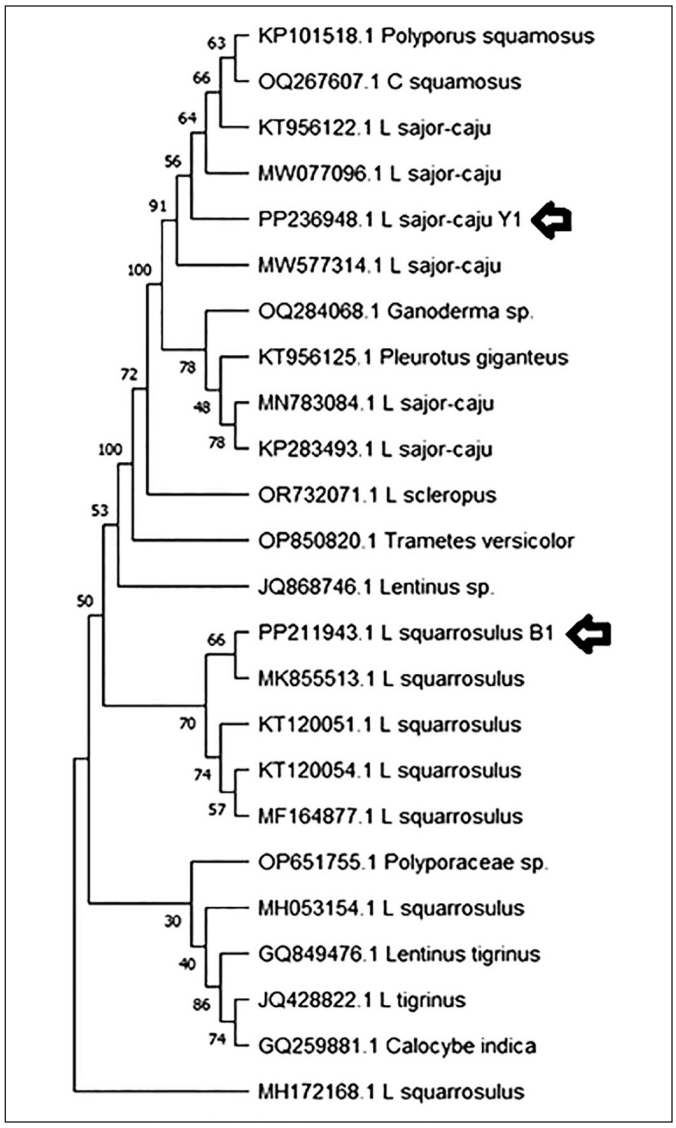

Figure 2. Phylogeny tree of fungi *L. squarrosulus* PP211943.1 (B1 sample) and *L. sajor-caju* PP236948.1 (Y1 sample).

Lentinus spp. are macrofungi distributed in tropical and subtropical regions and predominantly growing in tropical areas [29]. Despite their various benefits, efforts for their cultivation are still limited. Mushrooms are cultivated by SSF, which involves fermentation under sufficient humidity to support fungal growth and metabolic activities. This method aims to replicate the natural growth environment of isolated fungi as closely as possible [11]. SSF offers several advantages over submerged fermentation, including water and energy efficiency, ease of extraction, production of high-value products, and utilization of industrial waste substrates, making the former more economically viable [30]. This study selected papaya peel substrate due to its economic importance among the Caricaceae family and its widespread cultivation in tropical countries such as Indonesia [10]. Using papaya peel as a growth medium allows for the conversion of agro-industrial waste into value-added products [12]. *L. squarrosulus* and *L. sajor-caju* demonstrated robust growth on papaya peel medium, with distinct differences in mycelial colony growth.

3.2. ANTIMICROBIAL ACTIVITY

Papaya peel medium in SSF can provide a favorable environment for fungal mycelium growth because it has high contents of water, protein, and carbohydrates [31]. The extracts of LSQ and LSJ mycelia cultivated on papaya peel medium were tested for antimicrobial activity. The inhibition zone of the extracts against the tested microorganisms can be used to detect the strength of the compounds present in the sample in inhibiting microorganism growth [32].

LSQ exhibited potential antibacterial activity against *S. aureus, S. epidermidis,* and *P. acnes* with inhibition zone diameters of 3.37 ±**1** 0.15, 0.87 ± 0.80, and 5.33 ± 1.24 mm, respectively. However, it could not inhibit *S. hominis, P. aeruginosa,* or *E. coli*. This research is the first to report the antibacterial activity of LSQ against *P. acnes*, which has been overlooked so far. Our study found that LSJ did not inhibit all the tested bacteria. Meanwhile, the papaya peel extract (EP) inhibited the growth of S. aureus ATCC25923 and S. epidermidis with inhibition diameters of 3.00 ± 0.52 and 0.33 ± 0.57 mm, respectively. LSQ and LSJ did not have antifungal activity against *Candida albicans* (Table 1). A previous report showed that EP could not inhibit the growth of *E. coli, P. acnes, S. aeruginosa,* and *P. hominis* [33,34].

In this study, the extracts had higher antimicrobial activities against Gram-positive bacteria; Gram-negative bacteria possess an external

Table 1. Inhibition zone of papaya peel medium and extracts of *L. squarrosulus* and *L. sajor-caju* mycelia cultivated on papaya peel medium against six pathogenic bacteria.

Strain	Diameter of inhibition zone (mm)				
	EP	LSJ	LSQ	C (-)	C (+)
S. aureus ATCC 25923	3.00 ± 0.52^{b}	0^{a}	3.37 ± 1.15^{b}	0^{a}	18.93 ± 0.75^{c}
S. hominis InaCC B226	0^{a}	0^{a}	0^{a}	0^{a}	21.53 ± 0.72^{b}
S. epidermidis	0.33 ± 0.57^{a}	0^{a}	0.87 ± 0.80^{a}	0^{a}	22.07 ± 1.87^{b}
P. acnes ATCC 6919	0^{a}	0^{a}	5.33 ± 1.24^{b}	0^{a}	14.70 ± 1.83^{c}
P. aeruginosa ATCC 27853	0^{a}	0^{a}	0^{a}	0^{a}	9.97 ± 2.08^{b}
E.coli ATCC 25922	0^{a}	0^{a}	0^{a}	0^{a}	8.50 ± 4.72^{b}
C. albicans	0^{a}	0^{a}	0^{a}	0^{a}	13.17 ± 0.72^{b}

Notes:

EP : Papaya peel extract
LSJ : Extract of *L. sajor-caju* mycelia cultivated on papaya peel medium
LSQ : Extract of *L. squarrosulus* mycelia cultivated on papaya peel medium
C- : Negative control (DMSO 0.5%)
C+ : Positive control (Chloramphenicol)

membrane coated with lipopolysaccharides which can block the entry of toxic compounds into cells [35,36]. This finding is in accordance with a previous report, which stated the ethanol extract of *L. squarrosulus* exhibited higher antibacterial activity against Gram-positive bacteria (*S. aureus* and *B. cereus*) compared with Gram-negative bacteria (*P. aeruginosa* and *E. coli*) [7]. Our study showed that all the tested extracts did not have potential antifungal activity against *C. albicans*. However, Mossebo *et al.* [37] reported that the extracts of *Lentinus* spp. basidiomes have antifungal activity against *C. albicans*. This variation in bioactivity may be attributed to the use of mushroom samples in different stages that may produce different profiles of antifungal compounds [38,37].

Table 2. Qualitative mycochemical test on papaya peel medium, extract of *L. squarrosulus* and *L. sajor-caju* mycelia cultivated on papaya peel medium.

Sample	Mycochemical				
	Alkaloids	Flavonoids	Saponins	Tannin	Terpenoids
EP	+	–	+	+	+
LSQ	+	–	–	–	–
LSJ	–	–	–	–	+

Notes:
EP : Papaya peel extract
LSJ : Extract of *L. sajor-caju* mycelia cultivated on papaya peel medium
LSQ : Extract of *L. squarrosulus* mycelia cultivated on papaya peel medium

3.3. Bioactive Compounds of Mushroom Extracts

The bioactive compounds of the mushroom extracts were identified using a two-pronged approach: qualitative mycochemical testing and quantitative GC-MS. This comprehensive method revealed that EP contained a diverse range of bioactive compounds, including alkaloids, saponins, tannins, and terpenoids. Meanwhile, *L. squarrosulus* consisted of alkaloids, and *L. sajor-caju* contained only terpenoids (Table 2).

Alkaloids have health benefits such as antimicrobial and anti-inflammatory properties, and terpenoids are used as anti-inflammatory and anticancer agents. Tannins exhibit anti-tumour and antiviral activities [20]. Papaya peel comprises active compounds such as terpenoids, tannins, saponins, alkaloids, and steroids [39]. A previous study reported that the ethanol extract of *L. squarrosulus* basidiomes consists of alkaloids, steroids, terpenoids, saponins, and anthraquinones, and the hot water with submerged *L. sajor-caju* mycelium contains terpenoids, alkaloids, and saponins [4]. The differences in mycochemical contents can be due to several factors, including mushroom growth stages and extraction solvents, with the secondary metabolites commonly found in the basidiome samples [40]. According to the antibacterial results, the LSQ extract containing

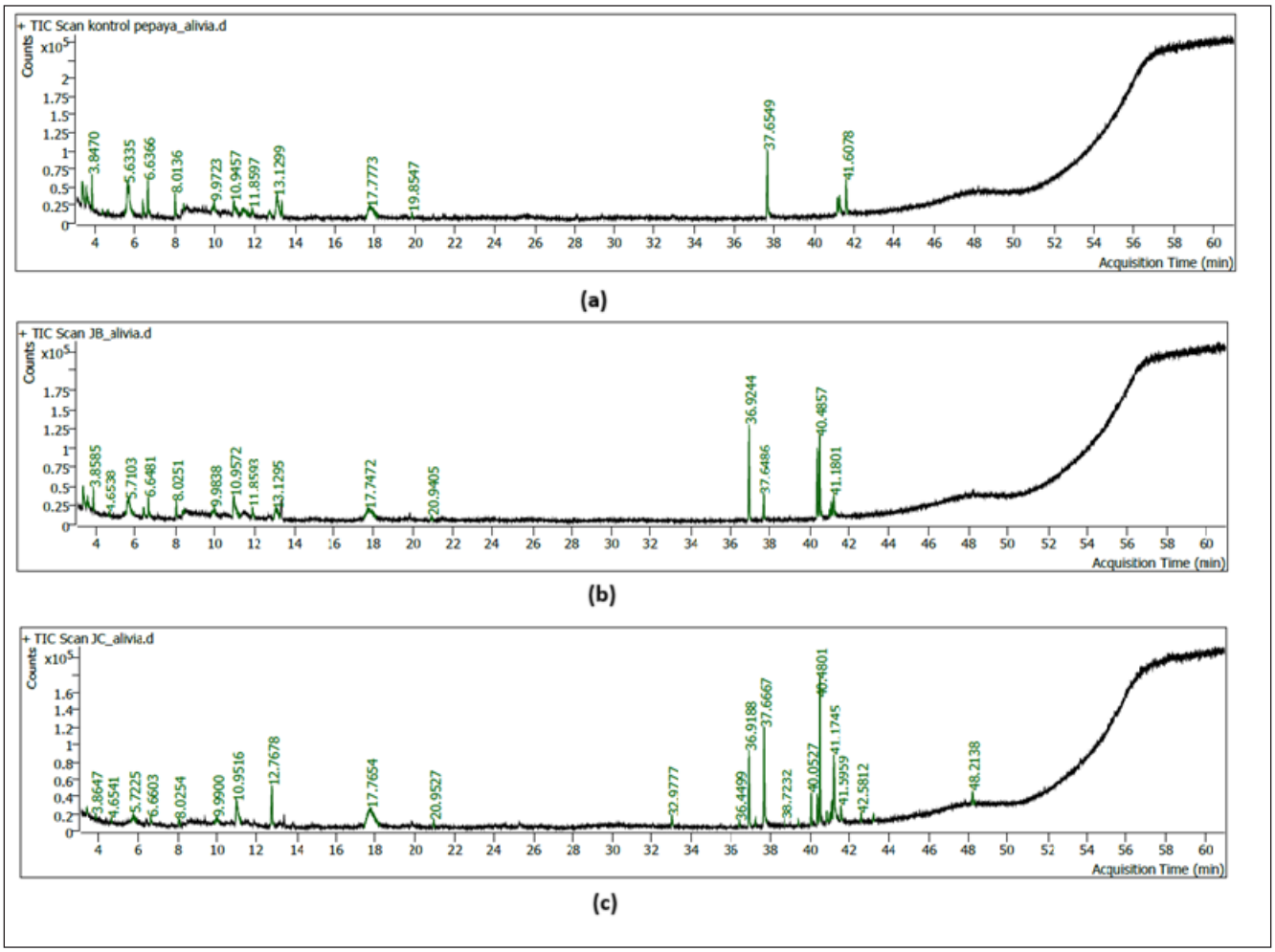

Figure 3. Chromatogram of GCMS analysis results (a) papaya peel extract. (b) *L. squarrosulus* (LSQ) extract. (c) *L. sajor-caju* (LSJ) extract.

Table 3. GC-MS results of papaya peel medium and extracts of *L. squarrosulus* and *L. sajor-caju* mycelia cultivated on papaya peel medium.

RT (minutes)	Compound name	Peak area (%)		
		EP	LSQ	LSJ
	Acid and derivatives			
3.562	Acetic acid, (acetyloxy)-	2.85	2.16	
3.847	Propanoic acid, 2-oxo-, methyl ester	2.72	1.56	
5.634	Propanoic acid, 3-nitro-, methyl ester	8.60	8.61	
9.972	Pentanoic acid	3.12		
32.978	Tetradecanoic acid			0.50
40.480	9-Octadecenoic acid, methyl ester, (E)-			5.52
41.174	cis-Vaccenic acid	3.39		3.72
41.608	Octadecanoic acid	4.57		0.63
	Benzene			
20.953	Thiocyanic acid, phenylmethyl ester			0.33
	Epoxides			
3.354	2,2'-Bioxirane		4.07	0.48
	Fatty acids and terpenoids			
37.655	n-Hexadecanoic acid	10.31	3.36	4.67
40.053	Methyl .gamma.-linolenate			1.20
40.367	9,12-Octadecadienoic acid (Z,Z)-, methyl ester		8.18	1.10
41.163	Oleic Acid	2.43		
	Furans			
17.777	5-Hydroxymethylfurfural	10.25	9.44	5.64
	Lactones			
6.387	Butyrolactone	2.07	1.38	
	Organooxygen compounds			
4.648	Furfural	0.48	0.51	0.13
	Organonitrogen compounds			
9.990	1-Hexanamine			0.67
	Oxazinanes			
9.984	Morpholine, 4-methyl-		1.90	
	Pyrans			
10.957	Maltol		8.73	
13.130	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.68	5.72	
	Unsaturated hydrocarbons			
36.450	1,12-Tridecadiene			0.23
40.830	1,8,11,14-Heptadecatetraene, (Z,Z,Z)-			0.48
	Others			
3.372	Butane, 1,2:3,4-diepoxy-, (.+/-.)-	4.68		
3.860	Ethyl acetoxycarbamate			0.14
4.360	2(5H)-Furanone, 3-methyl-	0.40		
5.699	4Cyclopentene-1,3-dione	7.52		0.76
6.637	1,2-Cyclopentanedione	4.67	2.47	
6.660	Cyclopentanone, 3-methyl-			0.28
8.014	2,4-Dihydroxy-2,5-dimethy-3(2H)-furan-3-one	2.49	1.85	0.30
8.346	1H-Pyrrole-2,5-dione		0.59	
8.447	2H-Pyran-2,6(3H)-dione	0.80	0.75	
10.946	Thymine	5.23		2.42
11.391	4,5-Diamino-2-hydroxypyrimidine	4.28		
11.860	N-Acetyl-3-pyrroline	1.37	1.96	
12.744	Ethanamine, N-ethyl-N-nitroso-	1.77		

Continued

RT (minutes)	Compound name	Peak area (%)		
		EP	LSQ	LSJ
12.768	Benzyl nitrile			1.93
20.940	3,5-Dimethyl-5-hexen-3-0l		0.98	
36.924	Hexadecanoic acid, methyl ester		10.85	2.75
37.245	E-10-Pentadecenol			0.23
38.723	Cyclohexane, 1-(cyclohexylmethyl)4-ethyl-,cis-			0.23
39.418	1,2-Diazaspiro(2.5)octane			0.20
40.486	9,12-Octadecadienoyl chloride, (Z,Z)-		10.82	
40.978	Methyl stearate		0.72	0.30
41.055	1,E-11,Z-13-Octadecatriene		1.73	0.81
41.180	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-		3.72	
42.581	E-11(130Methyl)tetradecen-1-ol			0.35
43.198	Cyclohexane,1-(cyclohexylmethyl)-4-ethyl-,cis-			0.28
48.214	Bis(2-ethylhexyl) phthalate			0.85

Notes:
EP : Papaya peel extract
LSJ : Extract of *L. sajor-caju* mycelia cultivated on papaya peel medium
LSQ : Extract of *L. squarrosulus* mycelia cultivated on papaya peel medium

alkaloids can inhibit the growth of *S. aureus, S. epidermidis,* and *P. acnes*. Alkaloids can inhibit bacterial growth by disrupting bacterial cell membranes, affecting DNA function, and inhibiting protein synthesis [41]. We found that EP contained alkaloids, saponins, tannins, and terpenoids, all of which may contribute to its antibacterial activity. Saponins could break down the structure of the cell membrane, allowing cell contents to flow out, and terpenoids cause cytoplasm coagulation [42]. Tannins could also deactivate enzymes, cell envelope protein carriers, and other proteins [43].

Table 3 lists more than 11 group compounds, including epoxides, organooxygen compounds, organonitrogen compounds, pyrans, furans, benzene, acids and their derivatives, lactones, oxazinanes, unsaturated hydrocarbons, fatty acids, and terpenoids, identified from the extracts. GC-MS results indicated that EP, LSQ, and LSJ contained 5-hydroxymethylfurfural, n-hexadecanoic acid, and 2,4-dihydroxy-2,5-dimethy-3(2H)-furan-3-one, with EP showing a higher peak area than the mushroom extracts (Fig. 3). Previous GC-MS testing did not detect these compounds, particularly in LSJ [44]. This finding suggested that the papaya peel medium used in the SSF might stimulate the production of these compounds in the cultivated mushrooms. These compounds may still be present in impure or crude form, so the resulting antibacterial activity is still low. This result is similar to the observations for *Aspergillus niger*, whose compound contents increased following SSF in rose flowers [45]. The 5-Hydroxymethylfurfural and n-hexadecanoic acid exhibit antibacterial activity against *S. aureus, B. substilis,* and *E. coli* [46]. The mode of action of 5-hydroxymethylfurfural might be related with its ability to bind with bacterial single-stranded DNA-binding protein. n-Hexadecanoic acid, which includes fatty acids and terpenoids, prevents the synthesis of important compounds, such as proteins, nucleic acids, and cell-wall components, disrupts cell membrane and bacterial DNA replication, and suppresses metabolic processes [47,48]. Additionally, these compounds possess anti-oxidant, anti-hypercholesterolemic, nematocidal, and pesticide activities [49].

GC-MS analysis of LSQ revealed its content of hexadecanoic acid methyl ester (10,85%), which was previously found in *L. squarrosulus* basidiome, 9,12-octadecadienoyl chloride (Z) (10,82%), and maltol (8,73%). Hexadecanoic acid methyl esters show promising antibacterial properties by modifying the structure of the cytoplasmic membrane and altering cell metabolism; as an antimicrobial agent, 9,12-octadecadienoyl chloride (Z,Z) could interact with water molecules, causing cell lysis and water loss due to osmotic disturbance [50,51]. Maltol may cause the shrinkage of the plasma membrane, leading to the degradation and lysis of bacterial cells [52]. These compounds from mushrooms were found using GC-MS, shedding light on their possible roles as active ingredients and their potential synergistic effects on the mushrooms' bioactivities, especially as antibacterial agents.

4. CONCLUSION

Two *Lentinus* species, *L. squarrosulus* and *L. sajor-caju*, were isolated from the Belitung Islands and Yogyakarta (Indonesia). LSQ exhibited antibacterial activity against *S. aureus, S. epidermidis,* and *P. acnes*, with moderate to weak impedance. Meanwhile, LSJ did not inhibit the tested bacteria. Qualitative mycochemical detection showed that LSQ contained alkaloids. GC-MS revealed hexadecanoic acid, methyl ester, 9,12-octadecadienoyl chloride, and maltol as the major bioactive compounds of LSQ.

5. ACKNOWLEDGMENT

The authors thank the Faculty of Animal Husbandry, Universitas Gadjah Mada, Indonesia for their assistance in GC-MS analysis.

6. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

7. FUNDING

The authors are grateful to the Doctoral Competency Improvement Program Universitas Gadjah Mada, Indonesia (No. 7743/UN1.P.II/Dit-lIT/pt.01.03/2023), for providing financial support for this research.

8. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

The data are available from the corresponding author upon appropriate request.

11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that no artificial intelligence (AI)-assisted technology was used in the writing or editing of the manuscript, and no images were manipulated using AI.

12. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

1. Kinge TR, Apalah NA, Nji TM, Acha AN, Mih AM. Species richness and traditional knowledge of macrofungi (mushrooms) in the awing forest reserve and communities, northwest region, Cameroon. J Mycol 2017;2017:1–9.
2. Suradkar KP, Kadu SR. Studies of some saprophytic fungi from Melghat Forest. Int J Sci Res Sci Technol 2021;9:254–7.
3. Izati N, Sugiyarto, Purwoko T. Diversity and distribution of macrofungi in pine forest and mixed forest in Mount Merbabu National Park. IOP Conference Series: Materials Science and Engineering, Bristol, UK, 2020, p 012030.
4. Fabros JA, Dulay RMR, De Leon AM, Kalaw SP, Reyes RG. Distribution, cultivation, nutritional composition, and bioactivities of *Lentinus* (Polyporaceae, Basidiomycetes): a review. Curr Res Env Appl Mycol 2022;12:170–219.
5. Hailong Y, Shen X, Chen H, Dong H, Zhang L, Yuan T, *et al.* Analysis of heavy metal content in *Lentinula edodes* and the main influencing factors. Food Control 2021;130:108198.
6. De Leon AM, Reyes RG, Delacruz TEE. *Lentinus squarrosulus* and *Polyporus grammocephalus*: newly domesticated, wild edible macrofungi from the Philippines. Philipp Agric Scientist 2013;96(4):411–8.
7. Sutthisa W, Chaiyacham P. Antibacterial activity of ethanolic extracts of *Lentinus squarrosulus* Mont. against human pathogenic bacteria. J Pure Appl Microbiol 2022;16:441–7.
8. Adeoye MO, Olajuyigbe OO, Gbolagade S, Coopoosamy RM. Bioactive compunds in ethanol extract of *Lentinus squarrosulus* Mont - a nigerian medicinal macrofungus. African J Tradit Complement Altern Med 2018;15:42–50.
9. Thomas L, Larroche C, Pandey A. Current developments in solid-state fermentation. Biochem Eng J 2013;81:146–61.
10. Saheed, OK, Jamal P, Karim MIA, Alam MZ, Muyibi SA. Utilization of fruit peels as carbon source for white rot fungi biomass production under submerged state bioconversion. J King Saud Uni-Sci 2016;28(2):143–51.
11. Behera S, Gupta N. Utilization of vegetable waste for biomass production of some wild edible mushroom cultures. Trop Plant Res 2015;2:5–9.
12. Pathak PD, Mandavgane SA, Kulkarni BD. Waste to wealth: a case study of papaya peel. Waste Biomass Valor. 2019;10(6):1755–66.
13. Watanabe T. Pictorial atlas of soil and seed fungi morphologies of cultured fungi and key to species. 2nd edition, CRC Press, Boca Raton, FL, 2002.
14. Putra IP. Guide for Indonesian macroscopic fungi characterization: part i – description of macroscopic characteristics. J Penelit Kehutan Wallacea 2021;10:25–37.
15. Panda MK, Das SK, Mohapatra S, Debata PR, Tayung K, Thatoi H. All aspects of plant biology mycochemical composition, bioactivities, and phylogenetic placement of three wild edible Russula species from Northern Odisha, India. Plant Biosyst 2021;155:1041–55.
16. Dutta A, Mahananda A, Adhikari S, Saha AK. Assessment of antibacterial activities of mycelium and exopolysaccharide extract of two different *Lentinus* species collected from Tripura. Vegetos, 2023;1–8.
17. Darmasiwi S, Aramsirirujiwet Y, Kimkong I. Evaluation of the nutritional value, mycochemicals, and antioxidant activities of *Hericium erinaceus* cultivated using jasmine rice. Asian J Agric Biol 2022;4:1–11.
18. Ushie VB, Onen OA, Ugbogu AI, Neji OC, Olumide PA. Phytochemical screening and antimicrobial activities of leaf extracts of *Swietenia macrophylla*. Chem Search J 2016;7:64–9.
19. Bharudin MA, Zakaria S, Chia CH. Condensed tannins from acacia mangium bark: characterization by spot tests and FTIR. AIP Conf Proc 2013;1571:153–7.
20. Aatika S, Zhang M, Wang Y, Zhu X, Liu X, Fan H, *et al.* Mycochemical screening and analysis, antioxidant activity, and biochemical composition of fermentation strain Snef1216 (*Penicillium chrysogenum*). J Anal Methods Chem 2020; 1:3073906.
21. Gneho DAD, Yeo D, Pakora AG, N'Guessan DJ. Evaluation of the antioxidant activity and mycochemical screening of two *Penicillium oxalicum* isolated from soil and leaves of *Solanum lycopersicum* respectively. Adv Biochem 2022;10:11.
22. Guntarti A, Pratiwi HK, Nurani LH, Gandjar IG. Authentication of lemongrass oil by gas chromatography-mass spectroscopy (GC-MS) combination chemometrics. Indo J Pharm Sci Technol 2022;9:174.
23. Panda MK, Das SK, Mohapatra S, Debata PR, Tayung K, Thatoi H. Mycochemical composition, bioactivities, and phylogenetic placement of three wild edible Russula species from Northern Odisha, India. Plant Biosyst 2021;155(5):1041–55.
24. Hermawan R, Sari AAP. *Lentinus sajor-caju* on the bases of morphological data. Biot J Ilm Biol 2021;19:75–9.
25. Fye LB, Abdullah N. Bioprospecting of *Lentinus squarrosulus* Mont., an underutilized wild edible mushroom, as a potential source of functional ingredients: a review. Trends Food Sci Tech 2017;61:116–31.
26. Senthilarasu G, The lentinoid fungi (*Lentinus* and *Panus*) from Western Ghats, India. IMA Fungus 2015;6:119–28.
27. Raja HA, Miller AN, Pearce CJ, Oberlies NH. Fungal identification using molecular tools: a primer for the natural products research community. J Nat Prod 2017;80:756–70.
28. Martin KJ, Rygiewicz PT. Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC Microbiol 2005;5:1–11.
29. Sysouphanthong P, Taliam W, Wannasawang N, Thongklang N. The genus *Lentinus* in Thailand: taxonomy, cultivation tests, nutritional analysis and screening for the biological activity of wild strains," Curr Res Environ Appl Mycol 2023;13:136–61.

30. Chen H. Modern solid state fermentation theory and practice. Springer Science Business Media, London, UK, 2013.
31. Pavithra CS, Devi SS, Suneetha JW, Durga Rani CV. Nutritional properties of papaya peel. Pharma Innov. J NAAS Rat TPI 2017;6:170–3.
32. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: a review. J Pharm Anal 2016;6:71–9.
33. Asghar N, Naqvi SAR, Hussain Z, Rasool N, Khan ZA, Shahzad SA, *et al.* Compositional difference in antioxidant and antibacterial activity of all parts of the *Carica papaya* using different solvents. Chem Cent J 2016;10:1–11.
34. Haneef M, Ceseracciu L, Canale C, Bayer IS, Guerrero JAH, Athanassiou A. Advanced materials from fungal mycelium: fabrication and tuning of physical properties. Sci Rep 2017;7:1–11.
35. Ebbensgaard A, Mordhorst H, Overgaard MT, Nielsen CG, Aarestrup FM, Hansen EB. Comparative evaluation of the antimicrobial activity of different antimicrobial peptides against a range of pathogenic Bacteria. PLoS One 2015;10:1–18.
36. Fengyu G, Chen Q, Liang Q, Zhang M, Chen W, Chen H, *et al.* Antimicrobial activity and proposed action mechanism of linalool against *Pseudomonas fluorescens*. Front Microbiol 2021;12:1–11.
37. Mossebo DC, Metsebing BP, Oba R, Tsigaing FT, Ryvarden L, Fonkui TY, *et al.* Comparative evaluation of antifungal and antibacterial activities of crude extracts of *Pleurotus sajor-caju, Pleurotus tuber-regium* and *Lentinus squarrosulus* (Basidiomycota, Pleurotaceae, Lentinaceae) from Cameroon. Eur J Biol Biotechnol 2020;1:1–7.
38. Dong C, Yang T, Lian T. A comparative study of the antimicrobial, antioxidant, and cytotoxic activities of methanol extracts from fruit bodies and fermented mycelia of caterpillar medicinal mushroom *Cordyceps militaris* (Ascomycetes). Int J Med Mushrooms 2014;16:485–95.
39. Siddique S, Nawaz S, Muhammad F, Akhtar B, Aslam B. Phytochemical screening and *in-vitro* evaluation of pharmacological activities of peels of *Musa sapientum* and *Carica papaya* fruit. Nat Prod Res 2018;32:1333.
40. Abdelghany TM. Mycology. OMICS Group eBooks, Foster City, CA, 2018.
41. Yan D, Li Y, Liu Y, Li N, Zhang X, Yan C. Antimicrobial properties of chitosan and chitosan derivatives in the treatment of enteric infections. Molecules 2021;26(23):7136.
42. Yu Z, Wu X, He J. Study on the antifungal activity and mechanism of tea saponin from *Camellia oleifera* cake. Eur Food Res Technol 2022;1:1–3.
43. Rahman MM, Rahaman MS, Islam MR, Hossain ME, Mannan Mithi F, Ahmed M, *et al.* Multifunctional therapeutic potential of phytocomplexes and natural extracts for antimicrobial properties. Antibiotics 2021 Sep 6;10(9):1076.
44. Sharma SK, Atri NS. Nutraceutical composition of wild species of genus *Lentinus* Fr. from Nothern India. Curr Res Env Appl Mycol 2014;4:11–32.
45. Leonardo S, Jesús MC, Paola MR, Alejandro ZC, Juan AV, Noé AC. Solid-state fermentation with *Aspergillus niger* GH1 to enhance polyphenolic content and antioxidative activity of Castilla rose (*Purshia plicata*). Plants 2020;9:1518.
46. Ganesan T, Subban M, Christopher DB, Kuppannan SB, Seedevi P. Structural characterization of n-hexadecanoic acid from the leaves of *Ipomoea eriocarpa* and its antioxidant and antibacterial activities. Biomass Conv Bioref 2022; 14:14547–58.
47. Lin ES, Huang CY. The inhibition activities of the fruit extract of *Plinia cauliflora* against melanoma cells and the single-stranded DNA-binding protein (SSB) from *Klebsiella pneumoniae*. Appl Sci 2023;13(19):11061.
48. Sumayya SS, Lubaina AS, Murugan K. Bactericidal potentiality of purified terpenoid extracts from the selected sea weeds and its mode of action. J Trop Life Sci 2020;1:10(3).
49. Siswadi S, Saragih GS. Phytochemical analysis of bioactive compounds in ethanolic extract of *Sterculia quadrifida* R.Br. ICoLiST AIP Conf Proc 2021;2353:1–8.
50. Ji QY, Wang W, Yan H, Qu H, Liu Y, Qian Y, *et al.* The effect of different organic acids and their combination on the cell barrier and biofilm of *Escherichia coli*. Foods 2023;12(16):3011.
51. Treesuwan K, Jirapakkul W, Tongchitpakdee S, Chonhenchob V, Mahakarnchanakul W, Tongkhao K. Antimicrobial mechanism of salt/acid solution on microorganisms isolated from trimmed young coconut. Microorganisms 2023;11(4):873.
52. Ziklo N, Bibi M, Salama P. The antimicrobial mode of action of Maltol and its synergistic efficacy with selected cationic surfactants. Cosmetics 2021;8(3):86.

How to cite this article:
Maysaroh AN, Witasari LD, Darmasiwi S. Identification, antimicrobial activity, and mycochemicals of *Lentinus* spp. cultivated in papaya peel medium. J Appl Biol Biotech. 2025;13(2):104-112. DOI: 10.7324/JABB.2025.213846